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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 063 002
A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 82301700.9

(22) Date of filing: 31.03.82

(51) Int. Cl.³: A 61 K 49/02
A 61 K 37/12
//(A61K37/12, 31/725)

(30) Priority: 02.04.81 US 250174

(43) Date of publication of application:
20.10.82 Bulletin 82/42

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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(54) **Radiolabelled peptides derived from crosslinked fibrin for locating thrombi and injectable compositions thereof.**

(57) Thrombi are located *in vivo* by administering a radiolabelled peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2 derived from cross-linked fibrin to a human or animal and externally detecting the radiation emitted by the radiolabelled peptide.

EP 0 063 002 A2

RADIOLABELLED PEPTIDES DERIVED FROM CROSSLINKED FIBRIN FOR LOCATING THROMBI AND INJECTABLE COMPOSITIONS THEREOF

The present invention relates to a method of locating thrombi in humans and animals, and more particularly to the use of a radiolabelled peptide derived from the degradation of crosslinked fibrin by plasmin as an imaging agent for locating thrombi in vivo.

Disorders of the blood clotting system are present in a significant fraction of the human population. The most common such disorder is the formation of thrombi, clots formed in a blood vessel or heart cavity that remain at the point of formation. Thrombi in heart vessels, for example, can restrict blood flow, resulting in myocardial infarction (death of heart muscle), one of the most severe forms of heart attacks.

In addition, parts of a thrombus or the entire thrombus can dislodge from its point of attachment and move through the blood vessels until it reaches a point where the passage is restricted. The resulting sudden blockage of blood flow is known as a thromboembolism. One part of the circulation system particularly subject to emboli formation is in the lungs, the first point at which main art ries divide into smaller arteries and capillaries aft r the heart has r ceiv d blo d fr m the venous system. A 1968

study of all hospital deaths showed that pulmonary emboli were present in 50% of patients who died at age 60, and in 64% of those who died at age 70. In the patients with emboli, an embolus was the major cause of death in 43% of the cases. Overall, over 700,000 cases of pulmonary emboli are detected in the United States every year and more than 90% of these emboli can be traced to deep vein thrombosis.

Accordingly, methods which enable thrombi to be detected are of great medical importance so that preventive measures, such as anticoagulant therapy or surgery, can be taken.

In recent years, human fibrinogen labelled with a radioisotope has been used for the detection of thrombi in the deep veins of the leg and in other parts of the body. Fibrinogen can be labelled with iodine-125 (U.S. Patent 3,933,996) or technetium-99m (U.S. Patent 4,057,617) and injected via a suitable carrier into a vein where it enters into clot (thrombus) formation. Activation of fibrinogen by the enzyme thrombin causes the release of fibrinopeptides (fibrin monomers), which polymerize to form a fibrin polymer that forms part of a clot or thrombus. When radiolabelled fibrinogen enters into clot formation, the radioactivity becomes localized and the thrombus can be located by ext rnal detection of the radiation.

Although the use of radiolabelled fibrinogen has constituted an advance in the location of thrombi, some problems still exist. Fibrinogen is taken up only by relatively fresh or still forming thrombi and hence may not be sufficiently localized in old thrombi (i.e. more than 1 day old) to allow effective external imaging. Accordingly, an imaging agent that would be taken up by both forming thrombi and previously formed thrombi is highly desirable. However, no such agent was known prior to the present invention.

Accordingly, it is an object of this invention to provide a method of locating both newly formed and previously formed thrombi.

It is another object of this invention to provide a method of locating thrombi by means of a radiolabelled imaging agent that will be selectively taken up by both newly formed and previously formed thrombi which could thereby be located by external measurement of emitted radiation.

These and other objects of the invention, as will hereinafter become more readily apparent, have been achieved by administering a radiolabelled peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2

to a human or animal, wherein said p ptid is selectively taken up by thrombi, and externally detecting radiation emitted by said radiolabelled peptide.

According to a first aspect of the present invention, there is provided a pharmaceutically acceptable radiolabelled peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2.

According to another aspect of the present invention, there is provided an injectable composition for locating a thrombus comprising a radiolabelled peptide of the invention and a pharmaceutically acceptable carrier or diluent suitable for intravenous injection.

According to a further aspect of the present invention, there is provided a peptide of the invention for use in locating a thrombus in a human or animal.

According to yet another aspect of the invention, there is provided an analogous process for the preparation of a peptide of the invention which comprises radiolabelling in manner known per se a p ptide select d from Fragment E_1 isolated from

cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2.

Fragments E_1 and E_2 are soluble degradation products released from cross-linked fibrin by the action of the enzyme plasmin. These fragments have been previously known and reported, but it was not known that they would be taken up by either fresh or previously formed clots or thrombi.

The relationship between fibrinogen and Fragments E_1 and E_2 can best be seen when considered in view of clot-forming and clot-breakdown biochemistry.

Human fibrinogen is a soluble plasma protein which is cleaved by the enzyme thrombin and forms insoluble fibrin, the network or matrix of a clot. The fibrin can be covalently cross-linked by Factor XIIIa to form a stabilized clot. Human cross-linked fibrin is degraded by the enzyme plasmin, thereby releasing characteristic degradation products (DD)E complex, Fragments DD and E, and a polymer remnants. The (DD)E complex is the primary soluble plasmin degradation product released from cross-linked fibrin. This complex is susceptible to further action of plasmin according to the following scheme:

cross-linked fibrin -- $(DD)E_1$-- $(DD)E_2$ -- DD + E_3.

The initial complex contains Fragments DD and

E_1. Upon further digestion Fragment E_1 is cleaved to Fragment E_2 without loss of the ability to bind to Fragment DD. Digestion of Fragment E_2 to E_3 results in dissociation of the complex. Therefore the terminal plasmin digestion products of cross-linked fibrin are Fragments DD and E_3. This pattern of digestion is consistant regardless of the plasmin to fibrin ratio; however, the rate of formation of the terminal products differs significantly with the plasmin concentration.

Preparation of various plasmin degradation products has been previously reported by two of the present inventors, Olexa and Budzynski, in Biochemistry 18, 991 (1979), and in J. Biol. Chem.254, 4925 (1979) which are hereby incorporated by reference. The basic process reported in these publications for the preparation an isolation of Fragments E_1 and E_2 begins with the formation of a fibrin clot from fibrinogen enriched with Factor XIII. The clot is hydrolyzed with plasmin and the resulting digest is centrifuged to remove large clot particles. The supernatant contains soluble degradation products, including the desired Fragments E_1 and E_2. The degradation products are separated according to molecular weight, preferably on an agarose gel bead column, to giv the (DD)E complex. Fragments E_1 and

E_2 are obtained from the purifi d (DD)E complex by incubation in a concentrated salt solution to cause dissociation of DD and E_1 or E_2 fragments followed by separation according to molecular weight, preferably by means of an agarose gel bead column. A detailed description of the process used is given in the Olex and Budzynski publications listed above.

Fragments E are the plasmic cleavage product of human cross-linked fibrin which contains the NH_2-terminal regions of all six polypeptide chains of fibrinogen. At least three species of Fragment E have been isolated and characterized, i.e. Fragments E_1, E_2, and E_3, of molecular weights 60,000, 55,000 and 50,000. The species are sequential degradation products and microheterogeneity of each species has been noted. Fragments E_1 and E_2 have the ability to bind to Fragment DD from cross-linked fibrin but do not bind with the DD-E complex, fibrinogen, or any of the plasmic degradation products of fibrinogen or of non-cross-linked fibrin.

In more recent investigations leading to the present invention, the inventors determined that Fragment E_1 would incorporate into a forming fibrin clot in an <u>in vitro</u> system. This was the first indication that the E_1 fragment would be taken up in a clot. Further investigations indicat d that

Fragment E_1 also became incorporated into preformed clots, formed from normal plasma and aged in serum for 2 hours.

Since Fragment E_1 was found to bind to both forming fibrin clots and to preformed, aged fibrin clots but not to bind to soluble fibrinogen or plasma proteins, the inventors recognized that this molecule can act as a tracer to locate in vivo thrombi. Fragment E_1 radioactively labelled with ^{123}I, ^{125}I, ^{131}I, ^{111}In, ^{99m}Tc or another appropriate isotope having gamma radiation suitable for external imaging, can be intravenously injected into a patient suspected of having a thrombus. Periodically areas of the patient's body would be imaged by a gamma camera or scanned with a rectilinear scintillation scanner. An alternative method for surveying the deep veins of the legs employs a hand-held scintillation probe used to take counts at a plurality of points along each leg.

In determining the suitability of Fragment E_1 as an in vivo imaging agent, several factors must be taken into consideration. An efficient tracer for the labelling of in vivo thrombi should have the following characteristics: (1) it should be easily labelled with a radioactive isotope to a high specific activity; (2) when injected systemically it should both incorporate

specifically and quickly into forming clots and bind to aged clots; (3) unbound material should be quickly removed from the circulation; (4) the material should not bind to fibrinogen or to other soluble plasma proteins; (5) the amount of bound material should decrease as the clot lyses; and (6) the material should be non-antigenic. Fragment E_1 meets all of these requirements.

Fragment E_1 contains approximately twenty tyrosine residues and about ten histidine residues, and therefore can easily be labelled with radioactive iodine, for example, by the chloramine-T, iodine monochloride, Iodogen (1,3,4,6 - tetrachloro-3α, 6α-diphenyl glycoluril) or lactoperoxidase methods. Radiolabelling with other isotopes can also be easily accomplished, for example, with ^{99m}Tc as described in Abramochi et al, U.S. Patent 4,057,617 which is hereby incorporated by reference. Very stable attachment of radioactive metal ions can best be accomplished by using a bifunctional chelating agent, i.e., a molecule containing a metal complexing group which could be attached to the peptide through a covalent linkage. An example of such a bifunctional chelating agent has been described by Krejcarek and Tucker in Biochem. Biophys. Res. Commun. 77: 581-585 (1977), which is h reby incorporated by referenc .

Fragm nt E_1 binds to both forming clots and to aged clots as shown herein. The biological half-life of human Fragment E_1 in rabbits is 1.4 hours compared to 49.3 hours for fibrinogen. It is quite probable that human Fragment E_1 would have a relatively short half-life in humans as well. Fragment E_1 does not bind to fibrinogen or any fibrinogen degradation products, but binds to aligned fibrin monomer molecules in a fibrin strand (Tables 1 and 2). Fragment E_1 does not bind to any soluble plasma proteins. Since Fragment E_1 can be cleaved to Fragment E_3 by plasmin, losing its binding capacity, the Fragment E_1 incorporated into a fibrin clot can be cleaved and released into the blood. The loss of radioactive Fragment E_1 from the thrombus parallels lysis of the thrombus. Finally, since Fragment E_1 may be derived from human fibrinogen, it is not likely to be a potent antigen. In conclusion, Fragment E_1 or any part of Fragment E_1 which contains the binding sites, would be an efficient tracer for the localization of <u>in vivo</u> thrombi.

Where the product is to be used in the treatment of human beings, the Fragment E_1 should preferably be isolated from human cross-linked fibrin, in order to minimize its antigenicity, but for other purposes animal cross-lined fibrin is suitable as a source of Fragment E_1.

Fragment E_1 may be injected intravenously in any suitable pharmaceutical carrier or diluent, either alone or in combination with other therapeutic or diagnostic agents. Suitable carriers or diluents are those which dissolve Fragment E_1 or hold it in suspension and which are not toxic to the extent of permanently harming the host organism. Preferred are non-toxic aqueous solutions of salts or non-ionic compounds such as sodium chloride or glucose, most preferably at an isotonic concentration. Other drugs may be present provided that they do not interfere with the action of Fragment E_1 as an imaging agent. Suitable amounts for combination are 5-95% labelled Fragment E_1 and 95-5% other drug or drugs. Particularly suitable are those substances normally injected with thrombus imaging agents, such as anticogulants, especially heparin.

Fragment E_1 may be injected into the blood stream at any convenient point, although injection upstream from and near to the site of the suspected thrombus is preferred.

Suitable amounts for injection depend on the specific radioactivity of the radiolabelled Fragment E_1 and can easily be determined by either calculation or simple experimentation. Radiolabelled Fragment E_1 should be administered in an amount

sufficient to be detected by gamma camera imaging or other external radiation detection means capable of locating the localized radiation present in the thrombus, such as autoradiography. In general, about 10μCi to 50mCi of radiation should be injected in order to achieve this effect in humans. The actual amount would depend upon the properties of the radionuclide used (e.g., physical half-life and energies of emitted gamma rays). In general, preferred amounts would be within 50 to 100% of the maximum allowable administered dose (limited by prevalent standards of safety) based on target organ and whole body radiation exposure in experimental subjects.

Analyzing by scintillation scanning or other external detection methods may begin within one hour after injection or may be delayed as many as three days. Better results are generally obtained between 6 and 18 hours after injection.

In terms of amount by weight of radioactive Fragment E_1 that is administered, no apparent lower limit exists except for the degree to which Fragment E_1 may be labelled with a radioactive isotope. There does not appear to be any upper limit except for those created by solubility if Fragment E_1 is isolated from the same species int which it is

injected. An upper limit is set for injections of Fragment E_1 from a different species by immune reactions, as is well known in the art and determinable by simple experimentation. If the specific radioactivity of the Fragment E_1 is known, and the desired radioactivity is known as previously described, the amount of Fragment E_1 injected can be easily calculated. For example, if the specific activity is 2μCi/mg, a 5 mg sample would contain 10 μCi of radioactivity.

The high thrombus-to-blood ratios obtained with radioiodinated Fragment E_1 in fresh and aged thrombi imply that radiolabelled Fragment E_1 may have great clinical significance. In addition to detecting thrombi in the veins of the legs, the principal use of radiolabelled fibrin Fragment E_1 labelled with a suitable imaging isotope (e.g. ^{123}I, ^{111}In, ^{99m}Tc) would be useful for detection of thrombi or emboli anywhere in the body, for example, in the brain in the case of stroke, in the heart in the case of myocardial infarction, and also for detection of pulmonary emboli, for which there is no specific test at the present time.

In addition, since Fragment E_2 also exhibits binding with clots and thrombi, Fragment E_2 may be used, as described above for Fragment E_1, as a

injected. An upper limit is s t for inj ctions of Fragment E_1 from a different species by immune reactions, as is well known in the art and determinable by simple experimentation. If the specific radioactivity of the Fragment E_1 is known, and the desired radioactivity is known as previously described, the amount of Fragment E_1 injected can be easily calculated. For example, if the specific activity is 2μCi/mg, a 5 mg sample would contain 10 μCi of radioactivity.

The high thrombus-to-blood ratios obtained with radioiodinated Fragment E_1 in fresh and aged thrombi imply that radiolabelled Fragment E_1 may have great clinical significance. In addition to detecting thrombi in the veins of the legs, the principal use of radiolabelled fibrin Fragment E_1 labelled with a suitable imaging isotope (e.g. ^{123}I, ^{111}In, ^{99m}Tc) would be useful for detection of thrombi or emboli anywhere in the body, for example, in the brain in the case of stroke, in the heart in the case of myocardial infarction, and also for detection of pulmonary emboli, for which there is no specific test at the present time.

In addition, since Fragment E_2 also exhibits binding with clots and thrombi, Fragment E_2 may be used, as described above for Fragment E_1, as a

thrombus-imaging ag nt. Since both E_1 and E_2 exhibit binding with cross-linked fibrin, it is likely that a peptide having an amino acid sequence intermediate between the sequences present in Fragments E_1 and E_2 would also exhibit binding and be useful as thrombi imaging agents. Such peptides can be formed by limited proteolytic cleavage of terminal amino acids from the various chains of Fragment E_1, and can be labelled with a radioisotope in the same manner as Fragments E_1 and E_2.

Having generally described the invention, a more complete understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLES

Purification of Fragment $E_1 - E_2$

Human cross-lined fibrin was digested with plasmin (6 units/g fibrin) for 24 hours at 37°C. Approximately 500 mg of the digest was gel filtered on a Sepharose CL-6B column (2.5 x 190 cm) in a buffer containing 0.05 M Tris-HCl, 0.028 M sodium citrate 0.1 M sodium chloride, 25 units/ml Trasylol (aprotinin), and 0.02% sodium azide, pH 7.8. Fractions containing the (DD)E complex were diluted with an equal volume of 6 M urea/0.05 M sodium citrate,

pH 5.5, and incubated at 37°C for 1 hour, then rechromatographed on a Sepharose CL-6B column (2.5 x 190 cm) in the above buffer. This procedure dissociated the (DD)E complex and allowed purification of the Fragment E species. Fragments E_1 and E_2 were collected together and separated by chromatography through a 0.6 x 6 cm DEAE - cellulose column. The elution solvent was a linear gradient of 0 to 0.5 M Na Cl in 0.01 M sodium carbonate buffer, pH 8.9. Fractions containing peptide were identified by absorbance at 280 nm. Alternatively, the Fragments E were separated by a preparative isoelectric focusing in a pH gradient 4 to 6 with a sucrose gradient stabilizer. Pooled fractions were collected and, ampholytes were removed by dialyzing the Fragments E against two 500-fold volumes of 1.0 M sodium chloride, two 500-fold volumes of 0.15 M sodium chloride, and four 500-fold volumes of distilled water, and the fragments were then freeze-dried.

Preparation of Radiolabelled Fragment E_1 - E_2

Purified Fragment E_1 or E_2 was labelled with radioactive iodine by the iodine monochloride method described by McFarlane in Biochem. J., 62, 135-143 (1956), which is hereby incorporated by reference. The labelled preparation contained 0.9 iodine atoms/Fragment E_1 molecule and had a specific radio-

activity of 0.5 μCi/mg. Fragments E_2 and E_3 were labelled by the same method. When higher specific activity was desired, as for the animal experiments the Iodogen method (1,3,4,6 - tetrachloro-3α-,6α-diphenylglycoluril) was employed to attach ^{131}I, or ^{123}I to Fragments E_1 and E_2. Use of Iodogen for iodinating proteins has been described by Fraker and Speck in Biochem. Biophys Res. Commun. 80: 849-857 (1978), which is hereby incorporated by reference. The labelled preparation in this case was trace labelled without carrier and had a specific radioactive of up to 2 mCi/mg.

Characeterization of Fragment E_1 - E_3

The amino acid sequence of Fragments E_1, E_2 and E_3 have been determined. Each Fragment E contains six polypeptide chains, two remnants from each of the Aα, Bβ, and γ chains of fibrinogen. The parameters of the Fragment E molecules are outlined in Table 1 based upon the known amino acid sequence of fibrinogen.

Binding Experiments

The ability of Fragments E_1 and E_2 to associate with or bind to fibrinogen and fragments of fibrinogen or fibrin was tested in a soluble system. Fragment E and the species to be tested were mixed in a 1:1 molar ratio, then analyzed on Tris-glycine

polyacrylamide (9%) gels. Fragments E_1 and E_2 bind only to Fragment DD but not to fibrinogen, Fragments X, Y, D or E (Table 2). This indicates that Fragment E binds only to the aligned D regions of Fragment DD, but not to the monovalent Fragment D domain of fibrinogen or fibrinogen derivatives.

To test this binding on a surface interface, Sepharose-insolubilized fibrinogen, fibrin monomer and a short oligomer of cross-linked fibrin was prepared. Fragments E_1 and E_2 did not bind to Sepharose-fibrinogen or Sepharose-fibrin monomer, but bound to the cross-linked fibrin oligomer (Table 3). This again indicates that Fragments E_1 and E_2 do not bind to fibrinogen or fibrin monomer but only to aligned fibrin monomers in a fibrin strand.

Table 1

Composition of the polypeptide chains of three species of Fragment E from human crosslinked fibrin based on the amino acid sequence of human fibrinogen

E_1	α 17-78
	α 17-78
	β 15-122
	β 15-122
	γ 1-62
	γ 1-62
E_2	α 17-78
	α 17-78
	β 15-121
	β 54-121
	γ 1-62
	γ 1-62
E_3	α 20-78
	α 24-78
	β 54-120
	β 54-120
	γ 1-52
	γ 1-52

Table 2

DEMONSTRATION OF BINDING BY THE FORMATION OF STABLE COMPLEXES

Test Material	Source	Treatment	E_1	E_2	E_3	DD	(DD) E
DD	Crosslinked fibrin	None	+	+	-	-	-
(DD) E	"	None	-	-	-	-	-
E_1	"	None	-	-	-	+	-
E_2	"	None	-	-	-	-	-
E_3	"	None	-	-	-	-	-
Fibrinogen		H	-	-	-	-	-
"		T	-	-	-	+	-
X (stage 1)	figrinogen	H	-	-	-	-	-
"	"	T	-	-	-	+	-
X (STAGE 2)	"	H	-	-	-	-	-
"	"	T	-	-	-	+	-
Y (stage 2)	"	H	-	-	-	-	-
"	"	T	-	-	-	+	-
D (stage 2)	"	H	-	-	-	-	-
"	"	T	-	-	-	-	-
D (stage 3)	"	H	-	-	-	-	-
"	"	T	-	-	-	-	-
D (stage 2)	Non-crosslinked fibrin	None	-	-	-	-	-
D (stage 3)	"	None	-	-	-	-	-
E (stage 2)	"	None	-	-	-	-	-
E (stage 3)	"	None	-	-	-	-	-
E (stage 2)	Fibrinogen	H	-	-	-	-	-
"	"	T	-	-	-	-	-
E (stage 3)	"	T	-	-	-	-	-
"	"	T	-	-	-	-	-
NDSK	"	H	-	-	-	-	-
"	"	T	-	-	-	+	-

The binding studies were done either in the presence to hirudin (H) at 10 ATU/mg protein or thrombin (T) at 20 NIH units/mg or in the absence of any of these agents. NDSK = NH_2 - terminal disulfide knot

+ = binding

- = absence of binding

Table 3

BINDING OF FIBRINOGEN AND FIBRIN DERIVATIVES TO INSOLUBILIZED FIBRINOGEN, FIBRIN MONOMER AND CROSSLINKED FIBRIN

Derivative	Amount of Protein Bound to Insolubilized					
	Fibrinogen		Fibrin Monomer		Crosslinked Fibrin	
	mg	nmoles	mg	nmoles	mg	nmoles
Fragment E_1	0	0	0	0	1.2	20.0
Fragment E_2	0	0	0	0	0.9	16.1
Fragment E_3	0	0	0	0	0	0
NDSK	0	0	0.05	1.0	0.04	0.81
NDSK (thrombin treated)	0.4	6.66	0.825	13.8	0.716	11.9

Incorporation of Fragm nt E_1 Into Fibrin Clots

Fragment E_1 was tested for the ability to incorporate into a forming fibrin clot in an in vitro system. Fragments E_1 and E_3, radioactivity labelled with 125-Iodine, were added to normal human plasma. Clotting was initiated by the addition of thrombin, then the clot was wound out onto a glass rod. The radioactivity in the clot and in the serum was measured. Each concentration of Fragment E was tested in triplicate. The mean value is shown in Table 4. A significant proportion of the Fragment E_1 became incorporated into the fibrin clot while the Fragment E_3 remained in the serum. Therefore, Fragment E_1 can bind to a forming fibrin clot.

Binding of Fragment E_1 to Preformed Plasma Clots

Plasma clots were formed from 0.5 ml of normal human plasma, suspended on a wire coil and aged in the serum for 2 hours. The 125-Iodine labelled Fragments E_1 or E_3 were added to the serum and incubation continued for 1 hour. The clots were washed five times in 0.5 ml of 0.15 M sodium chloride. The radioactivity in the serum, washes and in the clot was measured. The Fragment E_1 bound to the clot but Fragment E_3 did not bind (Table 5). The amount of Fragment E_1 bound to a preformed or aged clot was low r than the amount incorporated into a forming clot and proportional to the surface area of the clot.

Table 4

INCORPORATION OF FRAGMENTS E_1 AND E_3 INTO FORMING FIBRIN CLOTS

125-I Fragment E	Concentration E[a] (M)	Concentration Fibrinogen[a] (M)	% Incorporated[b]
E_1	7.2×10^{-9}	4.4×10^{-6}	72.1%
	3.6×10^{-9}	4.4×10^{-6}	44.3%
	1.8×10^{-9}	4.4×10^{-6}	35.4%
	0.9×10^{-9}	4.4×10^{-6}	30.4%
	0.45×10^{-9}	4.4×10^{-6}	32.8%
	0.275×10^{-9}	4.4×10^{-6}	30.4%
E_3	7.2×10^{-9}	4.4×10^{-6}	1.6%
	3.6×10^{-9}	4.4×10^{-6}	1.4%
	1.8×10^{-9}	4.4×10^{-6}	1.2%
	0.9×10^{-9}	4.4×10^{-6}	1.5%
	0.45×10^{-9}	4.4×10^{-6}	1.1%
	0.275×10^{-9}	4.4×10^{-6}	1.2%

[a] The initial concentration for fibrinogen and Fragment E are presented

[b] The percent of total radioactivity that remains with the compressed, washed clot, the mean of triplicate samples.

Table 5

BINDING OF FRAGMENTS E_1 AND E_3 TO A PREFORMED CLOT

125-I Fragment E	Concentration E[a] (M)	Concentration Fibrinogen[a] (M)	% Incorporated[b]
E_1	7.2×10^{-9}	4.4×10^{-6}	14.1%
	3.6×10^{-9}	4.4×10^{-6}	9.8%
	1.8×10^{-9}	4.4×10^{-6}	8.4%
	0.9×10^{-9}	4.4×10^{-6}	8.3%
	0.45×10^{-9}	4.4×10^{-6}	7.6%
	0.275×10^{-9}	4.4×10^{-6}	7.0%
E_3	7.2×10^{-9}	4.4×10^{-6}	0.13%
	3.6×10^{-9}	4.4×10^{-6}	0.11%
	1.8×10^{-9}	4.4×10^{-6}	0.19%
	0.9×10^{-9}	4.4×10^{-6}	0.21%
	0.45×10^{-9}	4.4×10^{-6}	0.21%
	0.275×10^{-9}	4.4×10^{-6}	0.13%

[a] The initial concentrations for fibrinogen and Fragment E are presented

[b] The percent of total radioactivity that remains with the compressed, washed clot, the mean of triplicate samples.

Incorporation of Radioiodinated Fragment E_1 Into Thrombi In Vivo

To test the potential of systemically injected radiolabelled Fragment E_1 for thrombus localization in humans with thrombosis, an in vivo model of thrombosis in animals was used. Because a thrombus is structurally heterogeneous (unlike clots), and because blood circulation and natural catabolic mechanisms can affect the uptake of tracers in vivo, these experiments were important in predicting the success of radiolabelled Fragment E_1 as a radiopharmaceutical for clinical thrombus localization.

Pigs were selected as the experimental animal model, as they are known to be quite similar to humans with respect to cardiovascular diseases, as described in Pond et al, "The Pig is a Model in Biomedical Research" in The Biology of the Pig, Comstock Pub. Assoc. pp. 31-35, 1978. Thrombi were induced in the jugular veins of young pigs weighing 25-50 lbs by a locally applied electric current. The method is known to produce thrombi which are morphologically similar to naturally occurring thrombi. After induction, the thrombi were allowed to age for up to 5 days prior to injection of radioiodinated Fragment E_1 into the pig. This permitted the study of tracer uptake in thrombi of various ages, ranging from very fresh

thrombi in which fibrin deposition is active, to aged thrombi in wich fibrin deposition is likely to be very low. In most cases, ^{125}I-labelled fibrinogen was injected simultaneously with the ^{131}I- or ^{123}I- labelled Fragment E_1, in order to directly compare the thrombus uptake of the two tracers. Radioiodinated fibrinogen is a tracer currently used for clinical detection of forming Deep Vein Thrombosis, and its thrombus uptake behavior has been well studied. Twenty-four hours after injection of the radiotracers, the thrombi were surgically removed and blood samples were drawn. The samples were weighed and counted.

The results of these experiments are listed in Table 6, for all ages of thrombi tested. A high target-to-background ratio is desirable in order to permit external imaging of a thrombus by a gamma scintillation camera. Because the main source of background radiation in thrombus imaging is likely to be due to blood pool radioactivity, the extent of localization in our experimental thrombi is expressed as a thrombus-to-blood ratio, which is defined as:

$$\frac{\text{Thrombus radioactivity per gram}}{\text{Blood radioactivity per gram}}$$

A thrombus:blood ratio of 4 is believed to be sufficient for imaging a thrombus in the veins of the

legs, and a ratio of 6 to 8 may be n cessary for imaging of thrombi in the chest. The results in Table 6 indicate that radioiodinated fibrinogen is appreciably localized only in very fresh thrombi (less than 20 hours old). Radioiodinated Fragment E_1, however, is localized to an impressive extent in thrombi of all ages tested (0-5 days). Because Fragment E_1 is thought to bind to the surface of a thrombus, the variation in uptake seen here may be due to differences in available thrombus surface area from animal to animal.

Table 6

THROMBUS UPTAKE IN PIGS
OF RADIOIODINATED HUMAN FRAGMENT E_1

Thrombus Age (hr)	FRAGMENT E_1 Thrombus:Blood Ratio	FIBRINOGEN Thrombus:Blood Ratio
1.25	10.4	41
4.4	10.0	18.8
5.6	108	16.9
20.5	9.5	2.8
23	17.5	3.9
	8.5	
24		
	40.5	
	9	0.5
	15	1.2
26	14.4	1.3
28.5	14	2.3
33	8.1	1.8
47	57	2.4
48	45	1.2
72	107	2.6
95	42	
120	18	

Having now fully described the inv ntion, it will be evident to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the scope of the invention as defined in the following Claims.

CLAIMS

1. A pharmaceutically acceptable radiolabelled peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2.

2. A peptide as claimed in Claim 1 which is labelled with a radioactive isotope of iodine, technetium, or indium.

3. A peptide as claimed in Claim 2 which is labelled with ^{111}In, ^{99m}Tc, ^{125}I, ^{131}I or ^{123}I.

4. A peptide as claimed in Claim 3 which is radiolabelled with ^{125}I or ^{99m}Tc.

5. A peptide as claimed in any one of the preceding Claims wherein said Fragment E_1 has an amino acid sequence identical to that of human fibrinogen amino acids α, 17-78; α, 17-78; β, 15-122; β, 15-122; γ, 1-62; and γ, 1-62 and wherein said Fragment E_2 has an amino acid sequence identical to that of human fibrinogen amino acids α, 17-78; α, 17--78; β, 15-121, β, 54-121; γ, 1-62; and γ, 1-62.

6. A peptide as claimed in any one of the preceding Claims for use in locating a thrombus in a human or animal.

7. An injectable composition for locating a thrombus, comprising a radiolabelled peptide as claimed in any one of the preceding Claims and a pharmaceutically acceptable carrier or diluent suitable for intravenous injection.

8. A composition as claimed in Claim 7 wherein said carrier is a non-toxic isotonic aqueous solution of a salt or a non-ionic compound.

9. A composition as claimed in Claim 8 wherein said carrier is a non-toxic isotonic aqueous solution of sodium chloride or glucose.

10. A composition as claimed in any one of Claims 7 to 9 wherein said composition further comprises an additional drug.

11. A composition as claimed in Claim 10 wherein said drug is an anticoagulant.

12. A composition as claimed in Claim 10 wherein said anticoagulant is heparin.

13. An analogous process for the preparation of a peptide as claimed in any one of Claims 1 to 7 which comprises radiolabelling in manner known per se a peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2.